

*H. A. Brown*

GRACE

Construction Products Division

APR 14 1976

CONSTRUCTION PRODUCTS DIVISION

CHARACTERIZATION AND PREPARATION  
OF RESPIRABLE SIZED TREMOLITE  
FIBER AND VERMICULITE  
FOR ANIMAL STUDIES

03630170

by: Julie C. Yang

April 8, 1976

10037750

## CAMBRIDGE

TO: H. C. Duecker

DATE:

April 8, 1976

FROM: Julie C. Yang

SUBJECT: Characterization and Preparation  
of Respirable Sized Tremolite  
Fiber and Vermiculite  
for Animal StudiesCC: H. A. Brown  
J. W. Wolter  
H. A. Eschenbach  
R. H. Locke  
File: 71-048

03630171

PURPOSE

The objectives of this study are to find out the size distribution and concentration of the respirable size fibers and vermiculite on the air filter collected by the the Industrial Hygiene and Environmental Health group in the field, and to prepare the samples corresponding as closely as possible to these air filter material, for animal studies.

AIR FILTER STUDY

Several randomly collected air samples from Libby at fairly long time intervals were collected for fiber contents and submitted to Arthur D. Little for sizing and distribution studies.

Two samples were sent:

<u>Sample No.</u>	<u>Collecting Time</u>	<u>Fiber Count (Optical/40 Fields)</u>
22260P-1	248 mins.	0.18 Fiber/cc air
22260P-2	300 mins.	2.15 Fiber/cc air

The results from Arthur D. Little are shown in Tables 1 and 2, Figures 1 - 3; and conclusions reached are summarized as follows:

- 1) On the air filter the respirable sized vermiculites and tremolite fibers are roughly in 50-50% ratio.
- 2) The respirable size tremolite fibers are mostly less than 10 microns ( $< 8\mu > 10\mu$  size), and the geometric mean length of the fibers is around  $3.1\mu$ .
- 3) The respirable size vermiculites are also less than  $10\mu$ , having an average size about  $5\mu$ .
- 4) The aspect ratio of the fibers is in the range of 11 to  $15\mu$ .
- 5) Computation shows that the fiber counting with SEM (scanning electron microscope) @ 20,000 magnification. The total numbers of fibers found per unit area ( $1\text{ cm}^2$ ) is about seven times in number of the fibers found by optical microscope counting at 400 magnification.

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To: H. C. Duecker  
From: J. C. Yang

Re: Animal Studies  
April 9, 1976

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SAMPLE PREPARATIONS

After we characterized what we have on the air filter, attempts were made to prepare both respirable sized vermiculite and tremolite fibers as closely as possible to those found on the air filter.

From previous research work (report on Libby Ore Evaluation - Ore Impurities, 2/23/76) we have found that Libby #2 vermiculite product has the highest tremolite fiber content in the order of 5% by weight. Since the sizes of #2 are fairly and easily to be handpicked, it is used as a starting source for both tremolite and vermiculite.

The tremolite fiber bundles picked out from Libby #2 are fairly clean and free of rocks, grayish in color, soft, and sometimes waxy in touch. They broke down easily to fine fibrils when degraded, which looked extremely similar to those found on the filter or floating in air in the Libby operation, which are quite different than the tremolite found in associated veins in rock form; they are generally harder and harsher, most of which were removed in the floatation process.

1) Tremolite Fibera) Cleaning

Tremolite fiber bundles were hand-picked from Libby #2 product, cleaned with acetone and then distilled water. The bundles were then opened with Waring Blender for 2 minutes at high speed, filtered and dried in the oven at 105°C. for about four hours.

b) Milling

The oven-dried material was Spec-milled in 0.5 g batch for a total of 45 seconds; but after each 10 seconds milling interval the mill was stopped and the material reruffled to avoid excessive packing.

The Spec-milled samples were then chilled in dry ice-acetone batch, chilling at low temperature increases the brittleness of the fibers and makes them easier to be pulverized. The chilled fibers were subjected to a Wiley mill with a built-in 60 mesh screen, a mill which has been designed especially for milling fibers. The Wiley milling was repeated another three times. Between runs the material has to be chilled again thoroughly with dry ice.

c) Sedimentation

0.8 g of the Wiley milled sample (mostly 2-4  $\mu$  in size, some up to 30  $\mu$  with some bundles under light microscope) was dispersed in two liters of distilled water, allowed to stand for 20 minutes; then, decant the cloudy solution into 250 ml or 500 ml graduated cylinders which were employed as sedimentation columns, and dilute the solution to twice its volume with distilled water. The solutions in each column were lightly stirred and allowed to settle for twenty minutes. The cloudy solution was then filtered by an HA type Millipore filter of 0.45  $\mu$ . However, the filtrate looked extremely clear and showed some small particles under the microscope.

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To: H. C. Duecker  
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The solid collected from the beaker and the column were recombined and treated with another 2 liters of distilled water, poured into columns and allowed to stand overnight. The cloudy solution was again decanted and filtered through the Millipore. Coarse solid remained at the bottom of the column from the second sedimentation, was filtered and saved for future remilling. The five fibers collected on the top of the Millipore were then examined by light microscope. It was found most of the particles were around 2  $\mu$ , and a few long fibers up to 20  $\mu$ .

d) Cleaning and Resizing

The finished crude product from step c was redispersed in the order of 2 g/4 liter distilled water, and allowed to stand in columns for over half an hour. The decanted cloudy solution (about twice as dense as solution in step c.) was then filtered through Millipore filter. The solid left at the bottom of the column was dispersed again, ultrasonically, for 2 minutes in 400 ml water. The milky solution was then diluted to another 4 liters and allowed to settle in columns for a final 20 minutes. The fines were collected on Millipore by filtering the decanted liquid, dried as examined by light microscope. The product has mostly 2  $\mu$  in size, very few larger fibers but a few up to 10  $\mu$ . The solid remained from decantation was again filtered and saved for future remilling.

2) Vermiculite

a. Cleaning

The vermiculite platlets were also hand-picked from Libby #2 product, cleaned in Soxhlet extractor with isopropyl alcohol, then acetone, and finally water to remove all the trace of organic contaminants used in the flotation process; then oven-dried at 105°C. for several hours.

b. Milling

The oven-dried vermiculite was then chilled with acetone and dry-ice mixture, Spec-milled in 2 g batches for 10 minutes. At the end of 5 minutes, the mill was stopped and the material was reruffled.

c. Screening

The milled sample was screened with 325 mesh screen. The -325 mesh product showed the desirable respirable size. Most of the particles were 2 - 4  $\mu$ . Some large plates were about 10 - 15  $\mu$ . The +325 mesh material was also collected and saved for future remilling.

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### 3) Proportioning

5 g of tremolite and 5 g of vermiculite, prepared from step 1) and 2) respectively, were carefully weighed out on a semimicro balance, and then transferred to a 4 oz. size wide-mouth glass bottle in which some silver wires were added to break up the powder surface when mixed on a roller mill. The mixing was carried out for about 16 hours. Because of the morphology and density difference, it will be suggested to Dr. Smith that when this sample is being used for animal study, an appreciable quantity (such as 1 or 2 grams) is taken, then dispersed in the saline medium ultrasonically, prior to use. The purpose of doing this will eliminate the localized inhomogeneity and selectiveness of a very small sample.

### 4) Characterization

The respirable-sized fibers (2260P-4 and 22250P-5) have been sent to A. D. Little for sizing and comparison with the fiber found on the air filter. The results are also shown in Tables 1 and 2, Figures 7 and 8. Scanning electron micrographs of these materials are shown in Figures 9 - 10.

Results from A. D. Little and our own microscopic sizing indicated that the respirable size fibers and vermiculite which we prepared are very similar to those on the air filter. However, sample 22260P-4 is a fiber sample of finer size, extremely time-consuming to obtain in large quantities. We have then taken a different approach to obtain 22260P-5 which is slightly coarser than 22260P-4. The two samples of 8 grams each we have submitted to Dr. W. Smith are:

1. 22260P-5 - respirable sized tremolite fiber
2. 22263P-2 - a mixture in 50-50% of respirable sized tremolite fiber (22260P-5) and vermiculite (22263P-1)

The final characterization of samples will be made by Walter McCrone Associates:

1. 22260P-5 respirable sized tremolite fiber
2. 22263P-1 respirable sized vermiculite
3. 22263P-3 a saline suspension of 22263P-2 will be prepared by W. Smith's group for animal studies.

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To: H. C. Duecker  
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April 9, 1976

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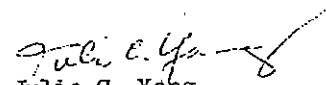
5) Sample Preparation for Animal Injection Studies

Dr. Smith's group has been preparing samples by dispersing 2 g of the solid in 40 ml 0.9 g saline solution in a 100 ml Erlenmeyer flask, then autoclaved for 15 minutes at 15 - 20 psi to sterilize the material. After it was cooled off, the mixture was shaken by hand and drawn into a syringe in 1 ml aliquot for injection.

By observing the preparations made with R. T. Vanderbilt sample (talc and tremolite mixture), solid settled very quickly in the saline solution immediately after shaking. Employing such technique, I would expect the animals got different doses of material depending on the technique of the operator and the rate of settling at that specific time. In addition, the fibers present may be in bundles or small balls not fully opened.

As a result, I have recommended the use of ultrasonic dispersion. The saline suspension after autoclaved should be subjected to a 10 minute sonic dispersion. It has been demonstrated the respirable sized material was suspended quite uniformly for an hour or more without settling. In case of any fiber balls or bundles present, they will be fully opened and dispersed, too.

Each animal will get 1 ml of the suspension which has 25 mg of the solid theoretically.

  
Julie C. Yang

JCY:mlr  
attachments

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TABLE 1

## SUMMARY OF LENGTH DATA

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Range (μ)	No. 1 (Total No.)		No. 2		22260-P4		22260-P5	
	N	Cum %	N	Cum %	N	Cum %	N	Cum %
<0.3	2	4	0	0	0	0	0	0
0.3-0.4	6	14	1	1	1	1	0	0
0.4-0.5	4	21	1	2	4	4	1	1
0.5-0.6	6	32	1	2	3	7	2	3
0.6-0.7	0	32	2	4	5	12	0	3
0.7-0.8	7	44	5	8	3	14	3	5
0.8-0.9	2	47	4	11	3	17	3	9
0.9-1.0	1	49	0	11	4	20	2	11
1.0-1.1	2	53	3	14	7	27	7	18
1.1-1.2	1	54	1	15	3	29	2	20
1.2-1.3	3	60	4	18	5	34	2	22
1.3-1.4	0	60	2	20	1	35	7	29
1.4-1.5	0	60	5	24	4	38	7	35
1.5-1.6	1	61	1	24	4	42	5	41
1.6-1.7	1	63	4	28	5	46	1	42
1.7-1.8	2	67	0	28	0	46	2	44
1.8-1.9	0	67	1	28	4	50	6	50
1.9-2.0	2	70	2	30	1	50	3	53
2.0-2.5	0	70	4	33	7	57	10	63
2.5-3.0	3	75	16	46	13	68	12	75
3.0-3.5	1	77	6	51	8	76	3	78
3.5-4.0	0	77	8	58	6	81	4	82
4.0-4.5	2	81	9	65	1	82	0	82
4.5-5.0	1	82	2	67	3	85	2	84
5.0-6.0	0	82	13	77	4	88	5	89
6.0-7.0	2	85	2	79	4	92	6	95
7.0-8.0	4	93	9	86	4	96	2	97
8.0-9.0	2	96	3	89	2	97	1	98
9.0-10.0	0	96	3	91	2	99	0	98
>10.0	2	100	11	100	1	100	2	100
Total	58		123		113		125	

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Arthur D Little Inc

TABLE 2

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SUMMARY DATA FROM A. D. LITTLE

Sample No.:	<u>22260P-1</u>	<u>22260P-2</u>	<u>22260P-4</u>	<u>22260P-5</u>
Total Fibers Counted	57	123	113	125
<u>Arithmetic Means</u>				
Length ( $\mu$ )	2.59	4.34	2.76	2.79
Width ( $\mu$ )	0.26	0.39	0.15	0.24
Average of Aspect Ratio	15.85	15.86	22.50	13.39
Mass ( $10^{-12}$ g)	0.5218	2.0464	0.1925	0.4982
<u>Geometric Means</u>				
Length ( $\mu$ )	1.38	3.11	1.97	2.07
Std. Deviation/Avg. Length	6.6	3.5	2.4	2.0
Width ( $\mu$ )	0.12	0.27	0.12	0.20
Average of Aspect Ratio	12.01	11.42	16.147	10.36
Mass ( $10^{-12}$ g)	0.0571	0.7162	0.0880	0.2584
Fibers/cm <sup>2</sup>	52,660	295,430		
Fiber Mass/cm <sup>2</sup> ( $10^{-9}$ g)	27.5	606.4		

J.C.Yang 4/8/76

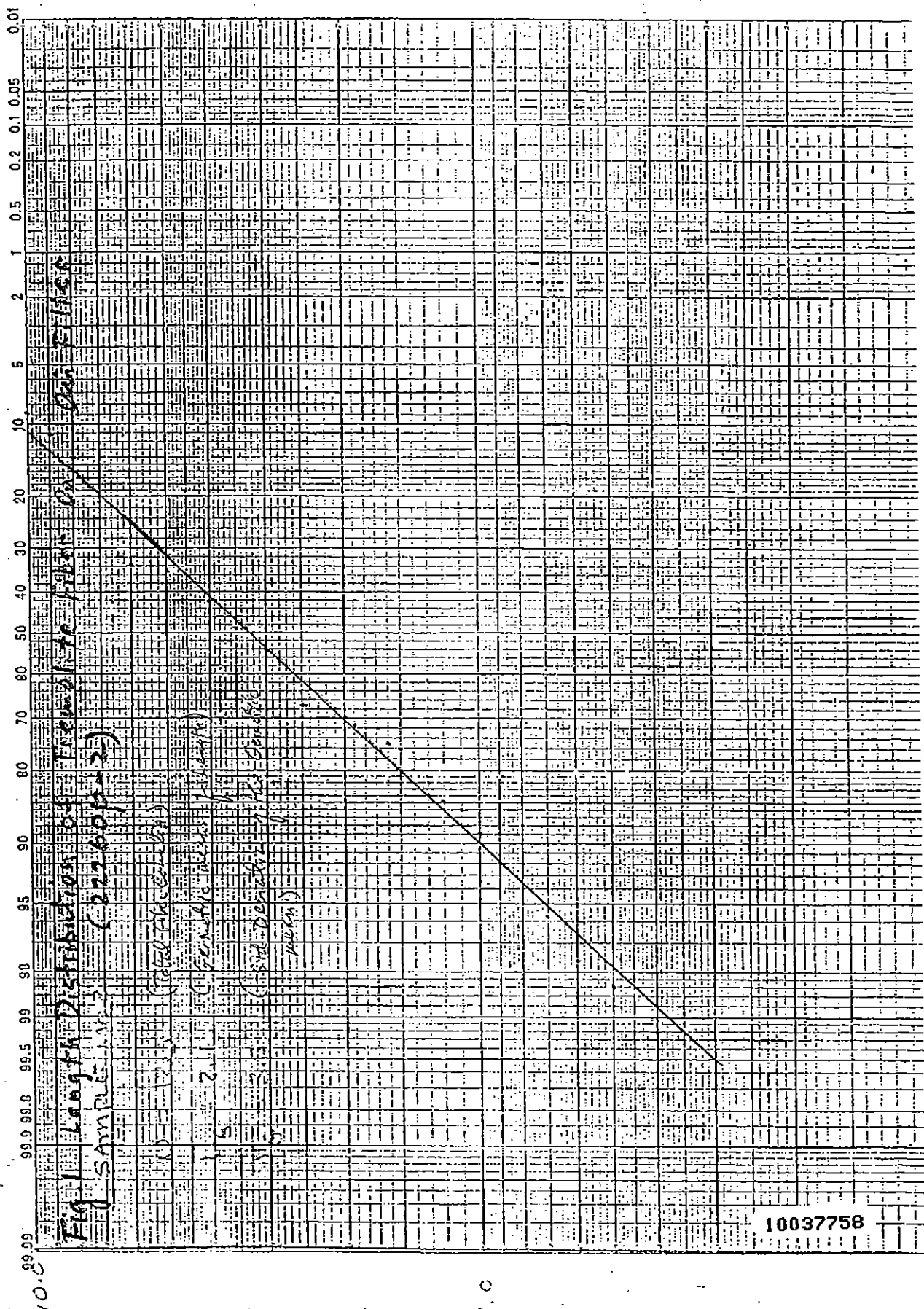
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NO. 3120. LOGARITHMIC PROBABILITY, DESIGNED BY HAZEN, WHIPPLE & FULLER.



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TREMOLITE PREPARATION

## TREMOLITE FIBER BUNDLES

(Handpicked from Libby #2 Product)

## CLEANING

Washing ↓ Acetone/H<sub>2</sub>O

Opening ↓ Waring Blender/hi speed/2 mins

filtering ↓

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drying ↓ oven / 105°C / 4 hrs

## MILLING

Spec-milling ↓ 45 sec / reruffle sample every 10 sec.

Freeze ↓ dry ice / acetone

Wiley milling ↓ repeat 4 times

disperse ↓ 1g solid / 2.5 L. dist. H<sub>2</sub>O

stand ↓ 20 min

decant ↓

Solid

Cloudy Soln

dilute ↓ 2X / dist. H<sub>2</sub>

stand ↓ 20 min

decant ↓

Solid

Cloudy Soln

filter Millipore  
0.45μDISPERSION  
±  
SIZING

redisperse ↓

1g / 2.5 L. distilled  
H<sub>2</sub>O

stand ↓

in columns  
20 min or over

decant ↓

Solid

(saved for  
future  
regrinding)

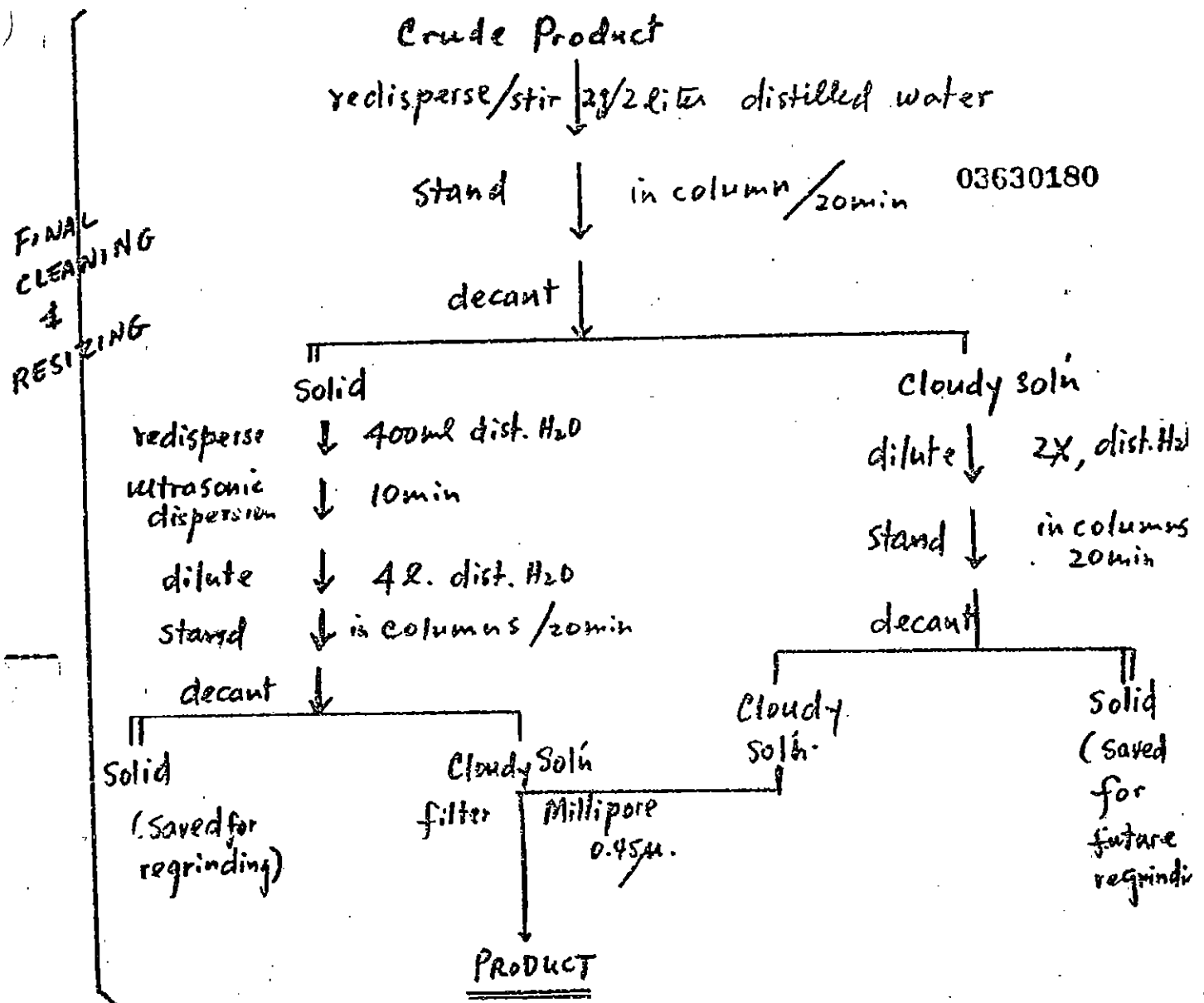
Cloudy liq.

filter Millipore, 0.45μ

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Crude Product

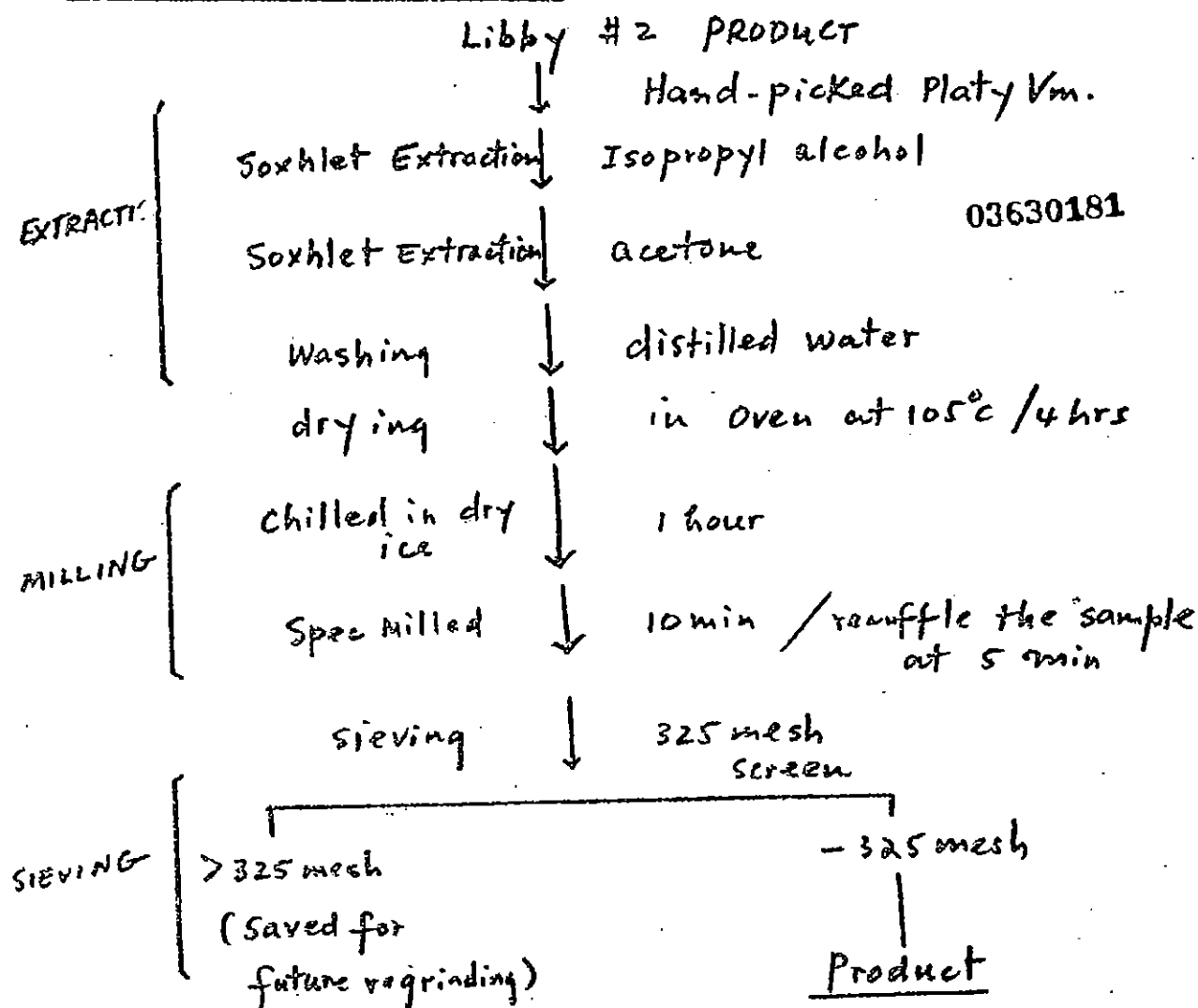
FIGURE 4 - TREMOLITE PREPARATION (Continued)

J.C. Yan  
3/23/76

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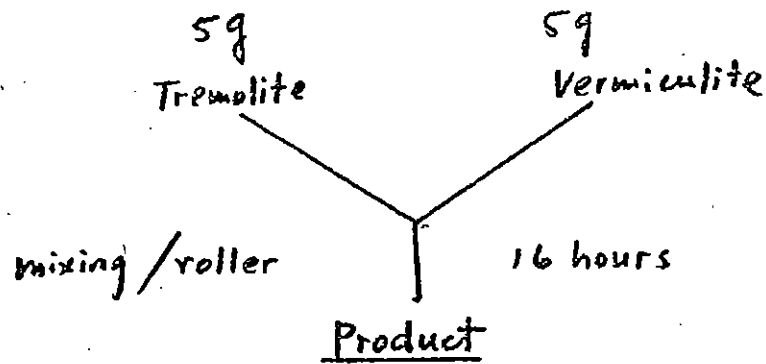
Fig. 5

VERMICULITE PREPARATION

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J.C. Yang / 3/24/03

Fig. 6  
PROPORTIONING



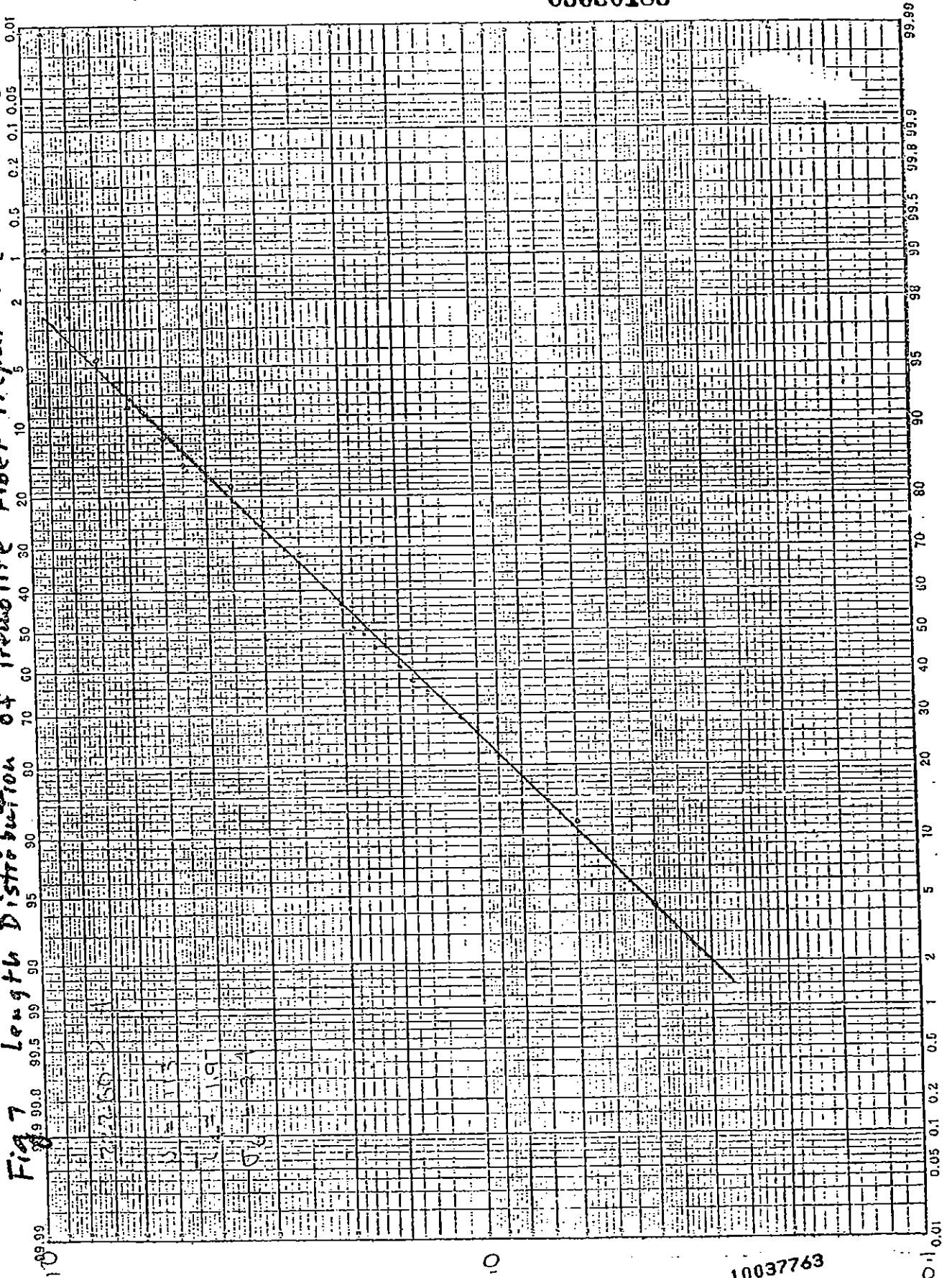
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Fig 7 Length Distribution of Tremolite Fiber Prepared (22260P-4)



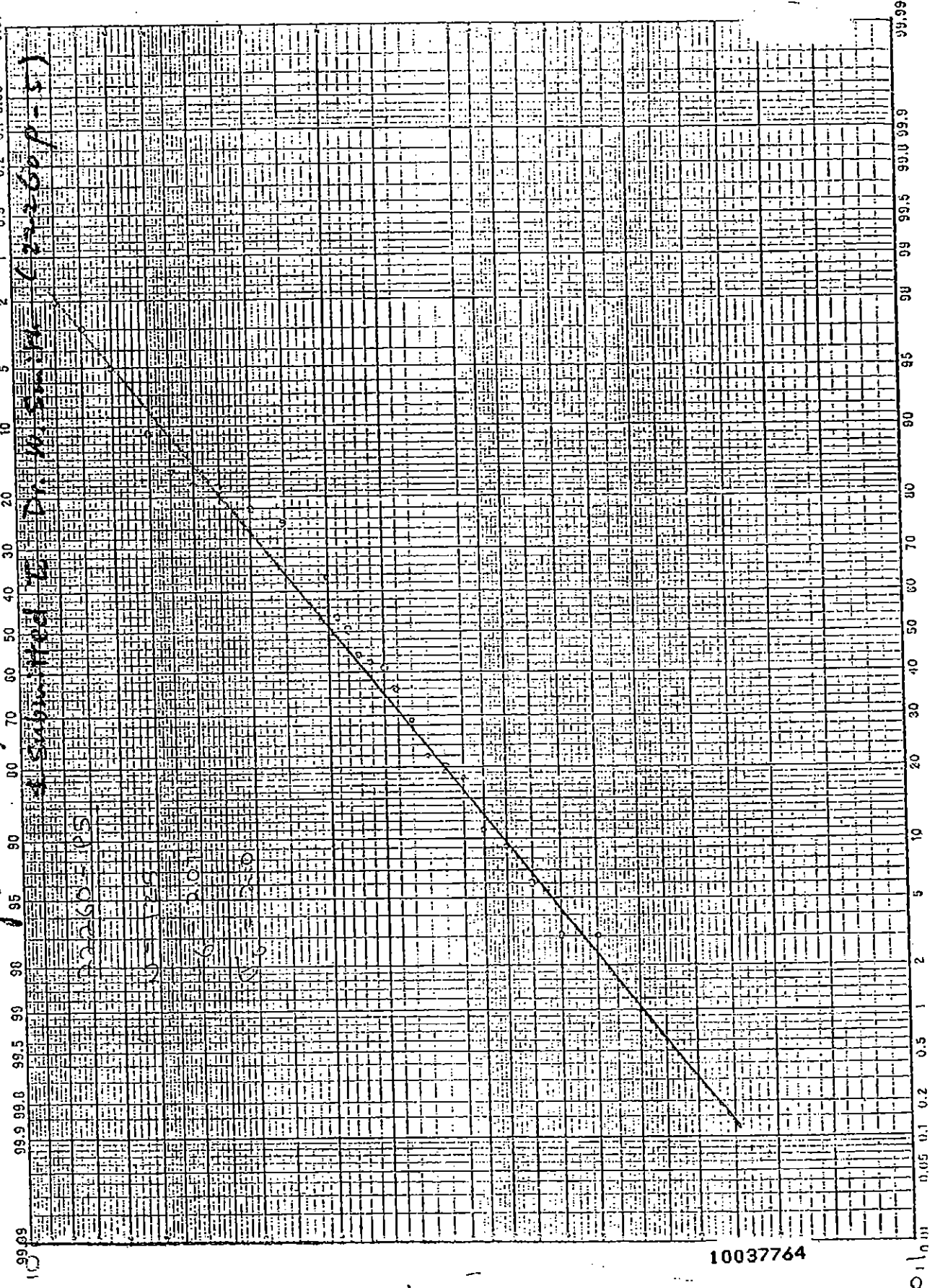
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Fig 8 Length Distribution of Tremolite Fiber Prepared



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ml - tustan

FIGURE 2  
SCANNING ELECTRON MICROGRAPHS (SEM)  
OF AIR FILTER #1

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2581-3

22260p-1

2400X

03630186



2581-5

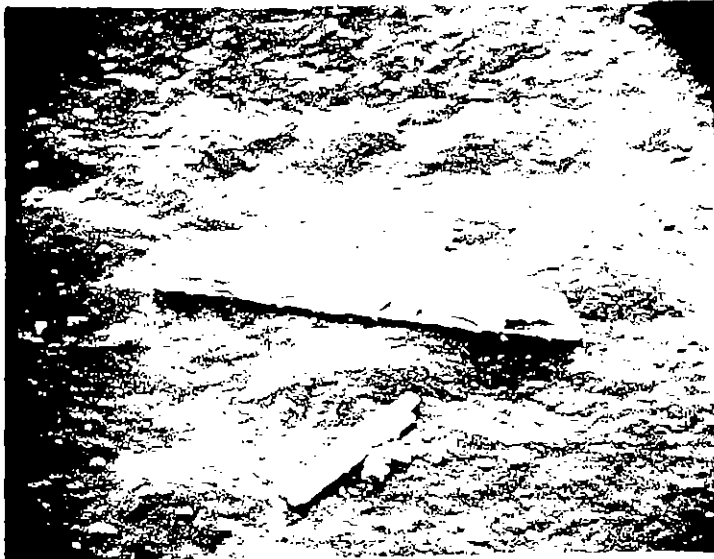
22260p-1

6000X

10037765



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2581-6

22260p-1

6000X

10037766

FIGURE 3  
SEM OF AIR FILTER #2

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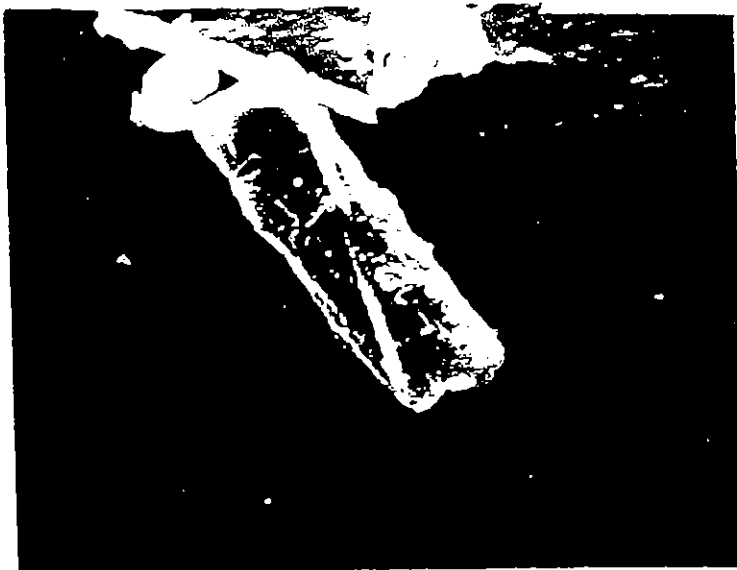


2582-4

22260P-2

2400X

03630189



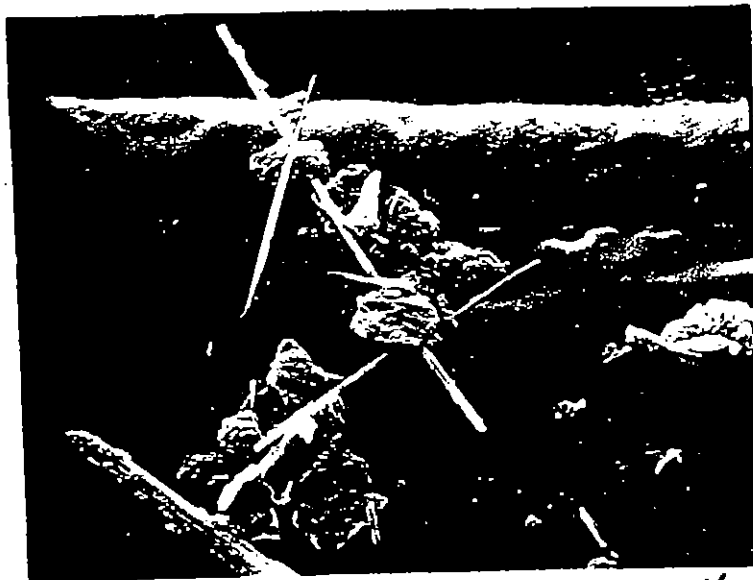
2582-2

22260-p-2

6000X

10037767

03630190

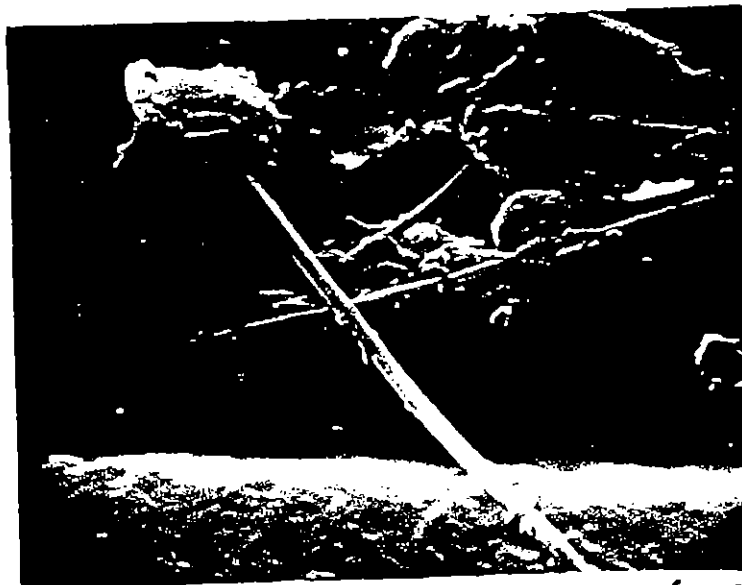


2582-6

22260p-2

2400X

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2582-2

22260p-2

6000X

10037768

FIGURE 9

SEM OF RESPIRABLE SIZE TREMOLITE  
FIBER PREPARED (22260P-4)

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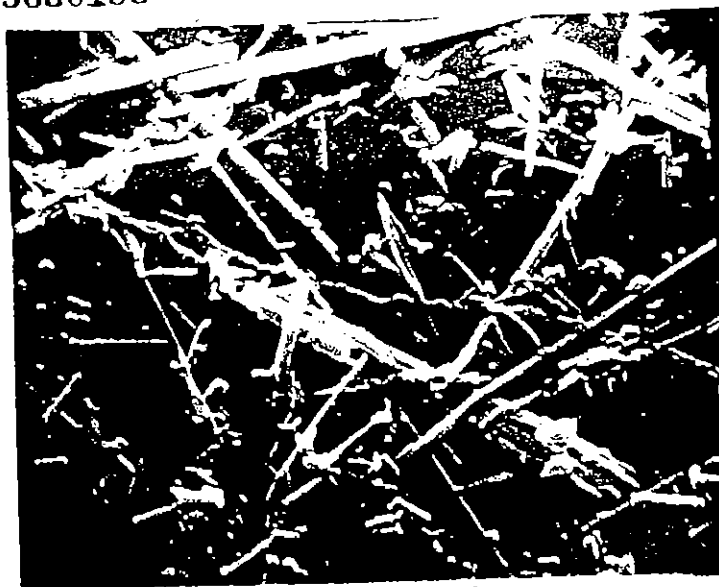


2607-5

22260p-4

2400X

03630193



2607-1

22260p-4

6000X

10037769



03630134

2607-5

22260p-4

6000X

10037770

FIGURE 10

SEM OF RESPIRABLE SIZE TREMOLITE FIBER  
PREPARED AND SUBMITTED TO DR. W. SMITH  
(22260P-5)

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2606-1 22260p-5 2000X

03630196



2606-3 22260p-5 2400X

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